Interactions between Surfactants in Solution and Electrospun Protein Fibers: Effects on Release Behavior and Fiber Properties

Boutrup Stephansen, Karen; García-Díaz, María; Jessen, Flemming; Chronakis, Ioannis S.; Nielsen, Hanne Mørck

Published in:
Molecular Pharmaceutics

Link to article, DOI:
10.1021/acs.molpharmaceut.5b00614

Publication date:
2016

Document Version
Peer reviewed version

Interactions between surfactants in solution and electrospun protein fibers - effects on release behavior and fiber properties.

Karen Stephansen[1,2], María García-Díaz[2], Flemming Jessen[1], Ioannis S. Chronakis[1*], Hanne M. Nielsen[2*]

[1]National Food Institute, Technical University of Denmark, Søltofts Plads 227, DK-2800 Kgs. Lyngby, Denmark.

[2]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

*Corresponding author:

Hanne Mørck Nielsen; mail: hanne.morck@sund.ku.dk, phone: +45 35 33 63 46, fax: +45 35 33 60 01, address: Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

Ioannis S. Chronakis; mail:ioach@food.dtu.dk, phone: +45 45 25 27 16, address: National Food Institute, Technical University of Denmark, Søltofts Plads 227, DK-2800 Kgs. Lyngby, Denmark

Abstract

Intermolecular interaction phenomena occurring between endogenous compounds such as proteins and bile salts and electrospun compounds applied for biomedical purposes is so far unreported, despite the exposure of fibers to such biorelevant compounds when applied for e.g. tissue engineering, wound healing and drug delivery. In the present study, we present a systematic investigation of how surfactants and proteins, as physiologically relevant components, interact with insulin-loaded fish sarcoplasmic protein (FSP) electrospun fibers (FSP-Ins fibers) and thereby affect fiber properties such as accessible surface hydrophilicity, physical stability and release characteristics of an encapsulated drug. Interactions between insulin-loaded protein fibers and five anionic surfactants (sodium taurocholate, sodium taurodeoxycholate, sodium glycocholate, sodium glycodeoxycholate, and sodium dodecyl sulfate), a cationic surfactant (benzalkonium chloride) and a neutral surfactant (Triton X-100) were studied. The anionic surfactants increased the insulin release in a concentration-dependent manner, whereas the neutral surfactant had no significant effect on the release. Interestingly, only minute amounts of insulin was released from the fibers when benzalkonium chloride was present. The FSP-Ins fibers appeared dense after incubation with this cationic surfactant, whereas high fiber porosity was observed after incubation with anionic or neutral surfactants. Contact angle measurements and staining with the hydrophobic dye 8-anilino-1-naphthalenesulfonic acid indiated that the FSP-Ins fibers where hydrophobic, and showed that the fiber surface properties were affected differently by the surfactants. Bovine serum albumin also affected insulin release *in vitro*, indicating that also proteins may affect the fiber performance in an *in vivo* setting.

Keywords: Electrospinning, proteins, surfactants, drug delivery, intermolecular interactions, tissue engineering

Introduction

Electrospun nanofibers may be applicable for a large range of biomedical applications within e.g tissue engineering, regenerative medicine and drug delivery[1–5]. The nanofibers can be functionalized in order to improve the performance *in vivo*, for example by addition of proteins to increase biocompatibility[6,7], addition of a polymer to strengthen the mechanical properties[7], addition of drugs such as antibiotics in wound dressing[8–10] or growth factors or DNA for tissue engineering[11–15]. Even cells can be electrospun to promote regeneration of new tissue[16–18]. During optimization of the fiber properties, special attention should be paid to the hydrophilicity of the fibers, fiber stability and drug release profiles (in case of encapsulated compounds)[12]. Drug release and stability properties are often studied *in vitro* in water[19,20], phosphate buffer[21], phosphate-buffered saline (PBS)[8,22–33] or other similar buffers not containing specific physiologically occurring compounds[34,35]. Depending on the application of the electrospun fibers, they will be exposed to different physiological environments, which contain compounds such as bile salts, proteins, and salts. However, the effects of physiologically occurring components on fiber stability and drug release characteristics are rarely taken into account, for which reason the fiber performance *in vitro* may deviate from the fiber performance *in vivo*. In a previous study we found that release of insulin from protein fibers was increased in simulated small intestinal fluid containing lipids and sodium taurocholate (ref; FSP-Ins paper). The study also demonstrated that transport of insulin across a Caco-2 cell monolayer was increased due to interactions between the cells and fibers. In a study by Alborzi *et al.* release of folic acid from alginate-pectin/polyethylene oxide electrospun fibers was studied in simulated intestinal fluid containing bile extract, and the authors found that the release was decreased in the presence of bile extract[36]. Sofokleous *et al.* studied release of amoxicillin from poly(D,L-lactide-co-glycolide) (PLGA) for wound healing purposes, and found the drug release from the PLGA dressings to be different in the

tested media (water, simulated body fluid and PBS)[10]. Moreover, Maretschek *et al.* found release of cytochrome C from poly(L-lactide) fibers to be increased when polysorbate was present in the release medium[37]. Despite of those observations, and to the authors knowledge, no study has been dedicated to detailed investigations on the potential effects in relation to the performance of electrospun fiber scaffolds caused by interactions with biorelevant compounds. In the current study, we investigated how biorelevant compounds affect the properties of electrospun fibers by systematically studying the interactions between insulin-loaded electrospun protein fibers and four different bile salts present in the human intestinal fluid (sodium taurocholate (TC), sodium taurodeoxycholate (TDC), sodium glycocholate (GC) and sodium glycodeoxycholate (GDC)). In order to further elucidate the mechanism accounting for the drug release induced by biological surfactants, different synthetic small molecular size surfactants were included in the study: the anionic sodium dodecyl sulfate (SDS), the cationic benzalkonium chloride (BC) and the neutral Triton X-100. The unpredictable effects of the different surfactants on the insulin release profile, fiber porosity and fiber surface properties are reported.

Materials and methods

Materials

Cod (*Gadus morhua*) from the North Sea was obtained from Hanstholm Fisk (Hanstholm, Denmark). Human insulin was kindly provided by Sanofi-Aventis Deutschland (Frankfurt, Germany). 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) was acquired from AppliChem (Darmstadt, Germany). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), 2-(N-morpholino)ethane sulfonic acid (MES), Hank´s balanced salt solution (HBSS), TC, TDC, GC, GDC, Triton X-100, SDS, 8-anilinonaphtalene-1-sulfonic acid (ANS), and benzoylacetone (BZA) were obtained from Sigma-Aldrich (St. Louis, MO,

USA), and BC was obtained from Nomeco (Copenhagen, Denmark). All other reagents were obtained commercially at analytical grade or HPLC grade for solvents for chromatography, and used without further purification. Ultrapure water from Barnstead NanoPure Systems (Thermo Scientific, Waltham, MA, USA) was used throughout the studies.

Preparation of FSP-Ins fibers

Fish sarcoplasmic proteins (FSP) were isolated from fresh cod and fibers were prepared as previously described[38] (Supplementary data S1, S2). A concentration of 125 mg/mL FSP was used together with 20 mg/mL insulin (Ins) for production of the FSP-Ins fibers. Electrospinning was conducted at room temperature, and samples were stored at -20°C until further analysis.

In vitro release studies

The *in vitro* release of insulin from FSP-Ins fibers was performed in HBSS buffer supplemented with 10 mM MES (MES-HBSS, pH 6.5) containing varying amounts (1.5 mM, 3 mM or 6 mM) of surfactant (TC, TDC, GC, GDC, Triton X-100, SDS or BC) during 5 h at 37°C with continuous orbital mixing. FSP-Ins fibers were placed in the pre-warmed buffers at a concentration of 3.5 mg/mL and aliquots of 30 µl were taken from the supernatant at regular time points and replaced with buffer. The samples were analyzed for contenct of insulin by using reverse phase high-pressure liquid chromatography (RP-HPLC) as described below. Each experiment was carried out at least in triplicates.

Insulin quantification

Insulin concentrations were analyzed by RP-HPLC using a Prominence system (Shimadzu, Kyoto, Japan) equipped with an Aeris WIDEPORE XB-C18 column (100×2.10 mm, 3.6 µm) (Phenomenex, Allerød, Denmark) and a PDA detector (SPD-M20A, Shimadzu). The mobile phase A consisted of 95%

H_2O/5% acetonitrile (AcCN)/0.1% trifluoroacetic acid (TFA) (v/v/v) and mobile phase B consisted of 95% AcCN/5% H_2O/0.1% TFA (v/v/v). The autosampler temperature was 4°C. Insulin was eluted using a linear gradient of mobile phase B from 20-50% over 3.5 min at a constant flow of 0.8 mL/min and with a column temperature of 40°C. The amount of insulin was quantified as the peak area obtained at 218 nm (retention time 2.7 min). Limit of detection and limit of quantification were 0.2 μg/mL and 0.8 μg/mL (n=3), respectively.

Internal fiber porosity

The internal porosity of FSP-Ins fibers was studied by using cryo-scanning electron microscopy (cryo-SEM) using a Quanta FEG 3D (FEI, Hillsboro, OR, USA) system equipped with a Leica cryo stage and the EM VCT100 Cryo transport system, in combination with the MED020 freeze fracture and Platinum/Carbon or Carbon coating unit (Leica, Wetzlar, Germany). The samples were incubated in MES-HBSS (pH 6.5) with 6 mM surfactant (TC, SDS, Triton X-100, BC), and subsequently placed on an aluminum stub that was mounted on a transport shuttle, and fixed to the stub with carbon/contact medium. The sample was frozen in liquid nitrogen in order to fracture the fibers with a cooled knife inside the MED020. Subsequently, the sample sublimated for 3 min and sputter-coated with platinum (coating thickness 5 nm).

Critical micelle concentration

The critical micelle concentration (CMC) of the different surfactants in MES-HBSS (pH 6.5) was determined according to the method described by Dominguez *et al.*[39]. Briefly, BZA was added to a final concentration of 0.14 mM, together with varying concentrations of each of the surfactant (TC, GC, TDC, GDO, Triton X-100, SDS and BC) in MES-HBSS. A total volume of 200 µl was added to a clear flat-bottomed 96-well UV-transparent plate (clear acrylic copolymer plate) (Corning Inc., Corning, NY,

USA), and absorption was measured at 313 nm at 37°C using a Safire^2 plate reader (Tecan Group, Männedorf, Switzerland).

Contact angle

The contact angle between FSP-Ins fibers and aqueous solutions of surfactants (6 mM TC, Triton X-100, SDS or BC in MES-HBSS, pH 6.5) was measured using an optical tensiometer (Theta, Attension, Espoo, Finland) with a highspeed camera (3000 fps, MotionXtra N3, IDT, Tallahassee, Fl, USA). A volume of 7.5 µl of buffer was deposited on the fiber surface, and images were immediately recorded with 59 frame per sec the first 3.4 sec, and thereafter 1 frame per second until 34 sec. The contact angle was derived from the images as the angle between the tangent to the drop at the surface-liquid interface and the fiber. The contact angle, calculated as the mean of the left and right contact angle, was plotted as a function of time, and the initial contact angle was found by extrapolation.

Confocal microscopy

FSP-Ins fibers were placed in MES-HBSS (pH 6.5) with and without surfactants (6 mM TC, Triton X-100 or BC) and incubated for 3 h. After 2 h, ANS was added to a final concentration of 25 µM and the incubation continued for 1 h. The fibers were transferred to a coverslip and images were obtained by using a laser scanning confocal microscope (Zeiss LSM 780, Jena, Germany) applying excitation at 405 nm and emission measured at 489 nm.

Statistics

Statistical analysis was performed using GraphPad (GraphPad, La Jolla, CA, USA). Unpaired Student's *t* test was used for assessment of statistically significant differences.

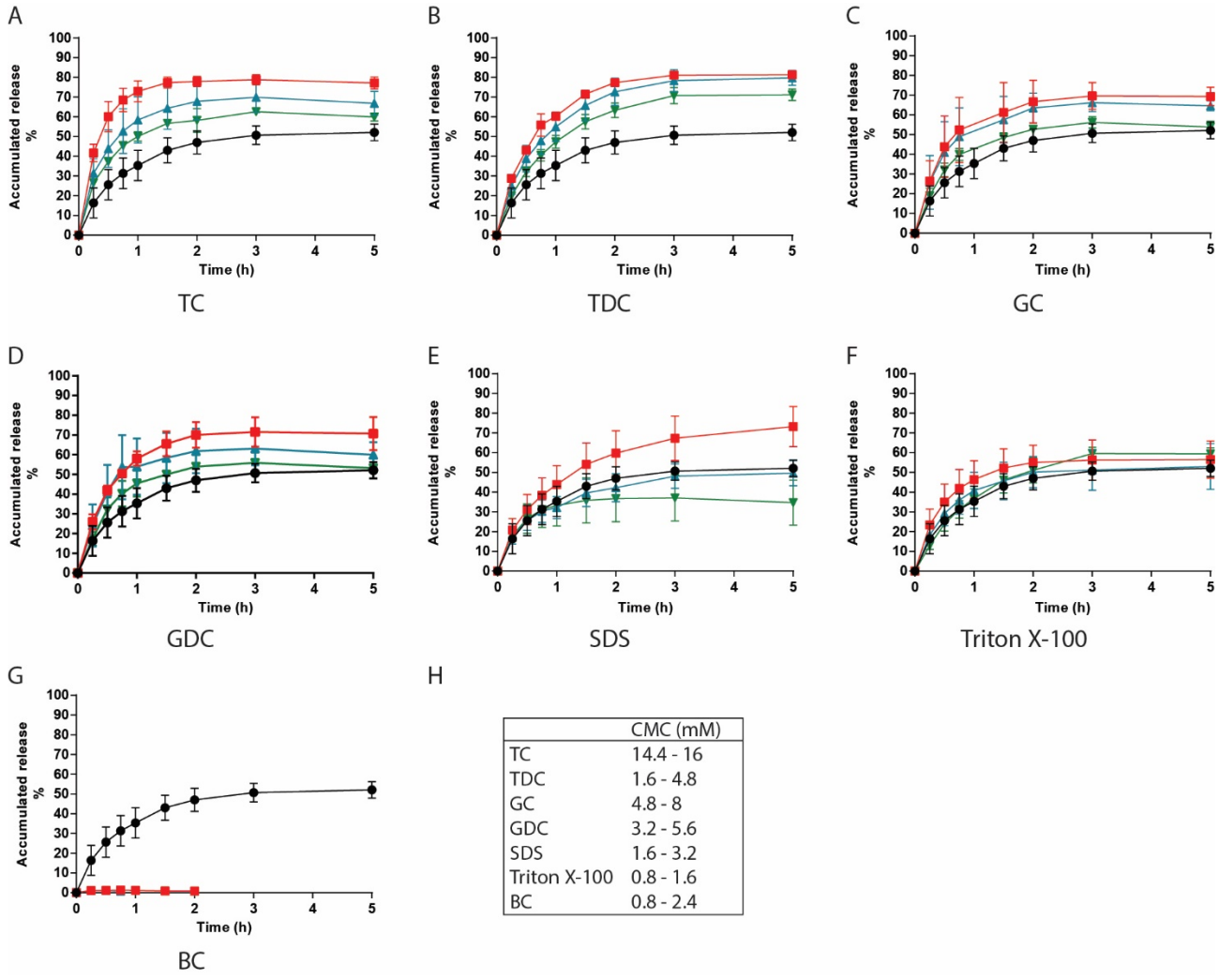
Results and discussion

Release of insulin from FSP-Ins fibers was affected by surfactants in solution

Insulin release from FSP-Ins fibers was studied in MES-HBSS in the absence or presence of surfactants (Figure 1). The chosen surfactants were divided into four groups, i) biologic anionic surfactants (TC, TDC, GC, GDC), ii) synthetic anionic surfactant (SDS), iii) cationic surfactant (BC) and iv) neutral surfactant (Triton X-100) (for the chemical structures see Supplementary information S3). The release was studied at increasing surfactant concentrations, up to 6 mM, and the presence of bile salts increased the release of insulin in a concentration-dependent manner (Figure 1 A–D). No additional effect on the release rate and the released amount of insulin was observed with bile salt concentrations higher than 6 mM (data not shown). The fibers were physically stable in the buffer during long periods of time, as no change in internal structure was observed even after 2 months of incubation (Supplementary data S4), and the fibers remained non-dissolved even longer. To investigate if the effect on the insulin release could be caused by surfactants in general, insulin release upon exposure to synthetic surfactants (SDS, BC and Triton X-100) was analyzed in a similar manner (Figure 1 E-H). SDS induced a similar behavior as the bile salts, with increasing SDS concentrations leading to increased insulin release. However, fibers that were exposed to high concentrations of SDS were degraded, as observed visually, and accordingly the release of insulin continued to increase, contrary to what was observed in the presence of the bile salts. The synthetic SDS is a well-known denaturing agent, and as a rule of thumb it takes 1.4 g SDS to denature a gram of protein to a completely unfolded structure. Although the FSP-fibers were not saturated with SDS at 6 mM SDS, degradation of the fibers clearly occurred. Further increasing the SDS concentration up to 18 mM led to complete release of the entrapped insulin due to extensive fiber degradation (data not shown). Complete drug release allowed for quantification of the insulin

encapsulation efficiency, which was found to be 98.6 ± 2.9 % (n = 3) (Ref FSP-Ins). Insulin release in 3 mM SDS was similar to the release in MES-HBSS, but it was noticeable that when lowering the SDS concentration to 1.5 mM SDS, the insulin release decreased. The presence of the neutral surfactant, Triton X-100, did not alter the insulin release profile as compared to that observed with the MES-HBSS (Figure 1F), and no concentration-dependency in the presence of SDS was observed. Interestingly, almost no insulin was released from the FSP-Ins fibers upon incubation with the cationic surfactant BC (Figure 1 G). Approximately 1 % of the encapsulated insulin was released in the 3 mM and 6 mM BC solution as burst release, but after 2 h no insulin was detectable. The strength of the interactions between BC and FSP-Ins fibers was investigated by transferring the FSP-Ins fibers to 12 mM SDS, after five h of incubation in 3 mM BC, and surprisingly no insulin was released after addition to the SDS solution (data not shown). This strongly indicated that the interaction between BC and the FSP-Ins fibers resulted in a significant stabilization of the fiber structure, preventing insulin release. The differences in the way the surfactants affected insulin release, suggested that i) the surfactants interacted differently with the FSP-Ins fibers, and ii) the interaction, and the results of the interaction, strongly depend on the properties of the surfactants. The CMC of the surfactants in MES-HBSS was determined to investigate if there was any relation between the CMC and the effect on release (Figure 1 H), however, no correlation to the CMC was found, which indicated that the organization of the surfactant molecules in solution did not affect the behavior of the surfactants with respect to fiber interactions. In our previous study, the simulated intestinal fluid also contained lipids, but the presence of lipids were found not to affect insulin release (data not shown). Bovine serum albumin (BSA) is a common protein in the human body, and for that reason the effect of BSA on insulin release was briefly touched upon. Boegh *et al.* have developed a biosimilar mucus, which contained 3.1 %(w/v) BSA, and with that amount of BSA in the MES-HBSS

buffer, the release of insulin was increased (Supplementary information S5) indicating that interactions with fibers and thereby alterating fiber properties is not limited to traditional small molecules surfactants.



	CMC (mM)
TC	14.4 - 16
TDC	1.6 - 4.8
GC	4.8 - 8
GDC	3.2 - 5.6
SDS	1.6 - 3.2
Triton X-100	0.8 - 1.6
BC	0.8 - 2.4

Figure 1. Insulin release from FSP-Ins fibers *in vitro* in MES-HBSS with varying concentrations of TC (A), TDC (B), GC (C), GDC (D), SDS (E), Triton X-100 (F) and BC (G); 0 mM (black), 1.5 mM (green), 3 mM (blue) and 6 mM (red). CMC values of the surfactants in the buffer is presented (H). Data represent mean ± SD, n ≥ 3.

Fiber porosity was affected by interactions with surfactant

In vitro release of a compound encapsulated in insoluble fibers has previously been correlated to the porosity of the fibers[19,20,40]. From those studies it was found that the release was facilitated by a two-step mechanism; desorption of the compound from the nanoporous surface of the fibers, as the rate limiting step, followed by diffusion from the fiber matrix into the buffer. As a result, only compounds situated at the surface of the fibers were released, as long as the fibers were intact. Modulating the porosity, and thus the surface area, for instance by addition of porogens, such as poly(ethylene glycol), or varying the polymer nature, concentration or molecular weight, modified the maximum released amount, and also the compound release profile [19,40,41]. In Figure 1the maximum release changed according to the type and the amount of surfactant present in the MES-HBSS buffer. To investigate if the observed tendencies were associated to changes in fiber porosity, the inner structure (cross-sections) of the FSP-Ins fibers after incubation in MES-HBSS, MES-HBSS with 6 mM TC or MES-HBSS with 6 mM BC, was analyzed with cryo-SEM, and compared to the inner structure of the fibers before incubation (Figure 2). Before incubation in a buffer, a small degree of porosity was observed throughout the fibers, and in some fibers the porosity in the core was increased, which most likely is caused by fast evaporation of the solvent (Figure 2D). After incubation in MES-HBSS and MES-HBSS with TC (Figure 2 A and B) the inner structure of the FSP-Ins fibers became more porous, whereas the fibers that had been exposed to BC became dense (Figure 2C). The trend in porosity correlated with insulin being released in MES-HBSS and MES-HBSS with TC, whereas release in MES-HBSS containing BC was negligible. The fact that the burst release in BC was almost nonexistent (Figure 1 G), suggested that BC instantaneously interacted with the surface of the fibers, and induced a stabilizing effect thus precluding insulin release and causing

the inner fiber structure to become dense. The inner structure of fibers exposed to Triton X-100 or SDS were also investigated (data not shown), and porosity was observed in both cases. As with MES-HBSS in the presence and absence of TC, porosity after exposure to Triton X-100 and SDS correlated with insulin being released from FSP-Ins fibers in both Triton X-100 and SDS solutions. It was not possible to study a potential connection between extend of insulin release and degree of porosity, since the porosity made the fibers fragile and sensitive to beam damage, which resulted in partly closing of the pores, which thereby prohibited quantitative analysis of the porosity.

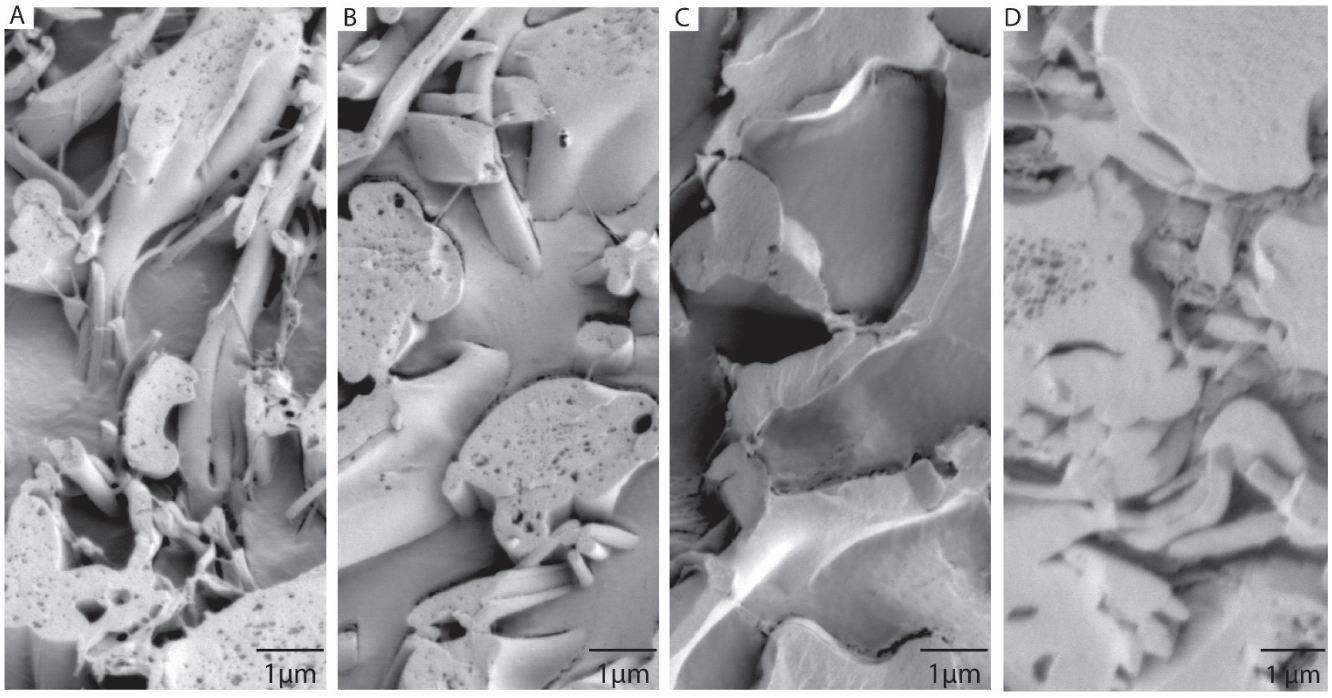


Figure 2. Cryo-SEM images of FSP-Ins fibers after incubation in MES-HBSS (A), MES-HBSS with 6 mM TC (B), MES-HBSS with 6 mM BC (C) and before incubation (D). Scale bars are 1 µm.

Interactions between surfactants and FSP-Ins fiber led to changes in the contact angles

The interactions between surfactants (TC, SDS, Triton X-100 and BC) in MES-HBSS and FSP-Ins fibers were further evaluated by contact angle measurements (Figure 3). MES-HBSS without surfactants

displayed a large contact angle of 110 ± 8 degr., which indicated that the FSP-Ins fibers were hydrophobic. Addition of SDS caused no significant change in the contact angle (117 ± 7 degr.), whereas addition of TC increased the contact angle to 130 ± 6 degr. The presence of BC decreased the contact angle to 84 ± 21 deg. And Triton X-100 decreased the value even further to 37 ± 13 degr. The decreased contact angle caused by Triton X-100 and BC suggested that interactions between Triton X-100, or BC, and the fibers, facilitated increased surface hydrophilicity. The rate by which the liquid drop was absorbed into the fibers, varied in the order Triton X-100 > BC > SDS > TC > MES-HBSS. The differences in contact angle and time for complete liquid drop absorption emphasized that the surfactants interacted with the fibers in different ways, dependent on the properties of the surfactants. The effect of the surfactant concentration was investigated by comparing the contact angle for 3 mM ans 6 mM TC or SDS, but no dose-dependency was observed in this concentration range (Figure 3 B)

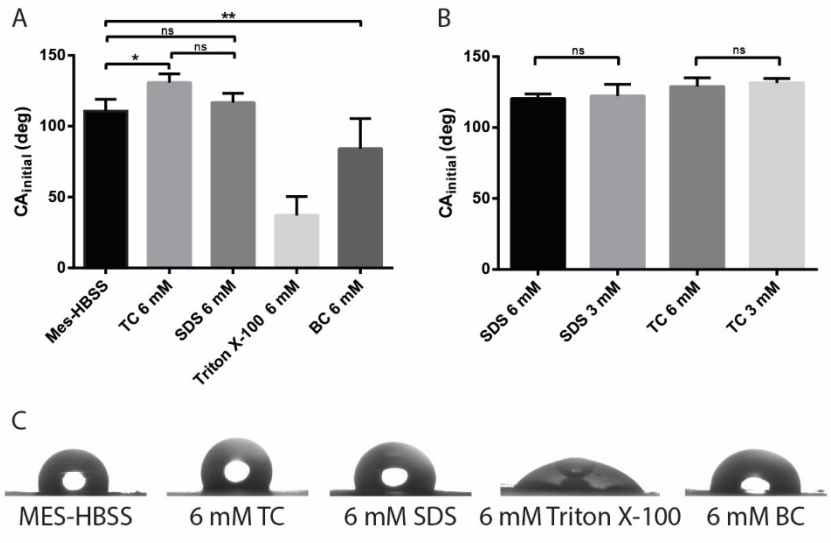


Figure 3. Contact angles between FSP-Ins and MES-HBSS with or without surfactants (A) and comparison of contact angles between FSP-Ins fibers and MES-HBSS containing either 3 mM or 6 mM surfactant (B). Data represent mean value ± SD, n > 3. Representative images of a drop of buffer (MES-HBSS with or without surfactants) on FSP-Ins fibers (C).

Surface properties of the FSP-Ins fibers were affected by interactions with surfactants

The hydrophobic nature of the FSP-Ins fibers was studied by ANS fluorescence (Figure 4). ANS is widely used to visualize hydrophobic regions in e.g. fibrillar aggregates and fibers, as its fluorescence is enhanced when ANS interacts with hydrophobic environments[42,43]. Increase in fluorescence intensity can thus be ascribed to the exposure of hydrophobic pockets to ANS molecules favouring the binding. Figure 4 A shows the ANS fluorescence in fibers after incubation with MES-HBSS. The visualization of the FSP-Ins fibers by the application of ANS fluorescence verified that the surface of the FSP-Ins fibers were hydrophobic. The homogeneity of the fluorescence throughout the fiber matrix furthermore suggested that the properties of the surface were consistent, despite of the complexity of the FSP matrix. The presence of 6 mM TC in the MES-HBSS buffer did not alter the ANS fluorescence (Figure 4 B), indicating that a similar amount of hydrophobic pockets were still available for interactions with ANS. Interesingly, esposure of the FSP-Ins fibers to 6 mM Triton X-100 or 6 mM BC caused a decrease in ANS fluorescence, as shown in Figure 4C and D, respectively. In both cases the ANS fluorescence decreased significantly, compared to fibers exposed to MES-HBSS without surfactants, indicating that the amount of accessible hydrophobic regions was decreased. This was in agreement with the contact angle measurements, where an increased hydrophilicity was caused by BC and Triton X-100, and hydrophobicity was observed for MES-HBSS in the presence and absence of TC. The loss in the amount of hydrophobic regions available for ANS binding can be explained by changes in the FSP-Ins fiber surface properties, and here we propose two possible explanations. In the electrospun fibers, the protein entanglements may cause the proteins to be in an energetically unfavorable state. Addition of surfactants to the buffer may cause a relaxation of the proteins, which results in small rearrangements in the protein structure, and thus shielding of hydrophobic regions. Another hypothesis rely on the strong interactions between surfactants and fibers, which will compete with and prevent the ANS molecules from interacting

with the fibers. Irrespective of the exact mechanism, changes in the surface properties of the fibers upon incubation with neutral or cationic surfactants were evident.

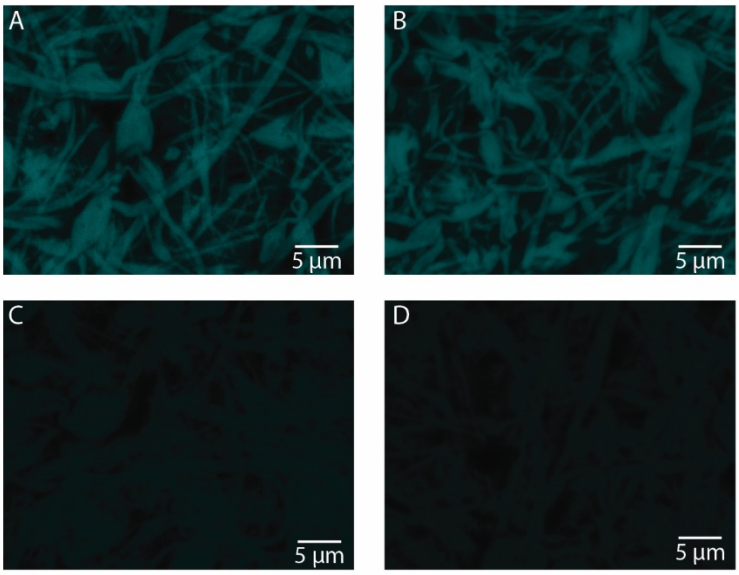


Figure 4. Cross-sectional confocal images of fibers displaying ANS fluorescence after incubation with FSP-Ins fibers in MES-HBSS (A), MES-HBSS with 6 mM TC (B), MES-HBSS with 6 mM Triton X-100 (C) and MES-HBSS with 6 mM BC (D). All images were recorded with the same settings.

Conclusion

The results presented in this study clearly indicate that biorelevant compounds interact with electrospun fibers. The interactions between surfactants in solution and electrospun protein fibers were found to significantly influence the release of entrapped cargo, the fiber stability, porosity and the fiber surface properties. Anionic surfactants were shown to have a concentration-dependent effect on insulin release, whereas addition of Triton X-100 did not cause a significant change in the amount released, and in MES-HBSS with BC, only negligible amounts of insulin were released. Cryo-SEM images of the fibers after incubation with the surfactants showed a correlation between release of cargo and increased porosity of the fibers, in the sense that when insulin was released, fiber porosity was observed. Contact angle

measurements revealed that the surfactants had a significant influence on wetting of the fibers; for anionic surfactants a small increase in the contact angle was observed, whereas the presence of the cationic surfactant, and especially the neutral surfactant, decreased the contact angle considerably. Interactions between fibers and surfactants were further elucidated, by decreased levels of interactions with the hydrophobic pocket fluorophore, ANS, after fiber incubation with neutral or cationic surfactants, as compared to anionic or no surfactants. Overall, this study emphasizes the importance of taking into account biologically relevant components, such as bile salts and proteins, with which electrospun fibers may be exposed to when used for tissue engineering, wound healing, drug delivery and other biomedical applications.

Acknowledgements

The authors would like to thank Dr. Vito Fodera for fruitful discussions about the surface properties of the FSP fibers. Nis Korsgaard Andersen and Dr. Raphael Taboryski is acknowledged for access to the optical tensiometer, and the Core Facility of Integrated Microscopy for assistance with cryo-SEM. Laboratory technician Karina Vissing is acknowledged for assistance with CMC determinations. Sanofi-Aventis is acknowledged for kindly supplying the human insulin. The research leading to these results has received support from the Danish Strategic Research Council (DSF -10-93456, FENAMI Project) and Innovative Medicines Initiative Joint Undertaking under grant agreement n°115363 resources, which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution.

References

1 T. J. Sill and H. a von Recum, *Biomaterials*, 2008, 29, 1989–2006.

2 Q. P. Pham, U. Sharma and A. G. Mikos, *Tissue Eng.*, 2006, **12**, 1197–1211.

3 D. B. Khadka and D. T. Haynie, *Nanomedicine Nanotechnology, Biol. Med.*, 2012, 8, 1242–1262.

4 D. B. Patel, R. Deshmukh, P. K. Pawde, S. Tadavi and R. V Kshirsagar, *J. Pharm. Res.*, 2009, **2**, 1184–1187.

5 F. Ignatious, L. Sun, C.-P. Lee and J. Baldoni, *Pharm. Res.*, 2010, **27**, 576–588.

6 P. Kuppan, S. Sethuraman and U. M. Krishnan, *J. Biomed. Nanotechnol.*, 2013, **9**, 1540–1555.

7 H. R. Pant and C. S. Kim, *Polym. Int.*, 2013, **62**, 1008–1013.

8 K. Kataria, A. Gupta, G. Rath, R. B. Mathur and S. R. Dhakate, *Int. J. Pharm.*, 2014, **469**, 102–110.

9 R. A. Thakur, C. A. Florek, J. Kohn and B. B. Michniak, *Int. J. Pharm.*, 2008, **364**, 87–93.

10 P. Sofokleous, E. Stride and M. Edirisinghe, *Pharm. Res.*, 2013, **30**, 1926–1938.

11 J. E. Trachtenberg, P. M. Mountziaris, F. K. Kasper and A. G. Mikos, *Isr. J. Chem.*, 2013, 53, 646–654.

12 W. Cui, Y. Zhou and J. Chang, *Sci. Technol. Adv. Mater.*, 2010, 11, 014108.

13 Y. J. Son, W. J. Kim and H. S. Yoo, *Arch. Pharm. Res.*, 2014, 37, 69–78.

14 Y.-F. Goh, I. Shakir and R. Hussain, *J. Mater. Sci.*, 2013, **48**, 3027–3054.

15 G. Jin, M. P. Prabhakaran, D. Kai and S. Ramakrishna, *Eur. J. Pharm. Biopharm.*, 2013, **85**, 689–698.

16 D. Poncelet, P. de Vos, N. Suter and S. N. Jayasinghe, *Adv. Healthc. Mater.*, 2012, **1**, 27–34.

17 E. Ehler and S. N. Jayasinghe, *Analyst*, 2014, **139**, 4449–52.

18 A. Townsend-Nicholson and S. N. Jayasinghe, *Biomacromolecules*, 2006, **7**, 3364–3369.

19 S. Khansari, S. Duzyer, S. Sinha-Ray, A. Hockenberger, A. L. Yarin and B. Pourdeyhimi, *Mol. Pharm.*, 2013, **10**, 4509–4526.

20 R. Srikar, A. L. Yarin, C. M. Megaridis, A. V Bazilevsky and E. Kelley, *Langmuir*, 2008, **24**, 965–974.

21 A. Y. A. Kaassis, N. Young, N. Sano, H. A. Merchant, D.-G. Yu, N. P. Chatterton and G. R. Williams, *J. Mater. Chem. B*, 2014, **2**, 1400.

22 D. Brahatheeswaran, A. Mathew, R. G. Aswathy, Y. Nagaoka, K. Venugopal, Y. Yoshida, T. Maekawa and D. Sakthikumar, *Biomed. Mater.*, 2012, 7, 045001.

23 F. Han, H. Zhang, J. Zhao, Y. Zhao and X. Yuan, *J. Biomater. Sci. Polym. Ed.*, 2013, **24**, 1244–59.

24 C. Hu, S. Liu, Y. Zhang, B. Li, H. Yang, C. Fan and W. Cui, *Acta Biomater.*, 2013, **9**, 7381–7388.

25 Y. Wang, B. Wang, W. Qiao and T. Yin, *J. Pharm. Sci.*, 2010, **99**, 4805–4811.

26 H. Jiang, Y. Hu, Y. Li, P. Zhao, K. Zhu and W. Chen, *J. Control. Release*, 2005, **108**, 237–243.

27 H. Jiang, D. Fang, B. Hsiao, B. Chu and W. Chen, *J. Biomater. Sci. Polym. Ed.*, 2004, **15**, 279–296.

28 M. Jannesari, J. Varshosaz, M. Morshed and M. Zamani, *Int. J. Nanomedicine*, 2011, **6**, 993–1003.

29 U. E. Illangakoon, T. Nazir, G. R. Williams and N. P. Chatterton, *J. Pharm. Sci.*, 2014, **103**, 283–292.

30 L. K. MacRi, L. Sheihet, A. J. Singer, J. Kohn and R. a F. Clark, *J. Control. Release*, 2012, **161**, 813–820.

31 J. Li, R. Fu, L. Li, G. Yang, S. Ding, Z. Zhong and S. Zhou, *Pharm. Res.*, 2014, **31**, 1632–1643.

32 J. Hu, J. Wei, W. Liu and Y. Chen, *J. Biomater. Sci. Polym. Ed.*, 2012, 24, 1–14.

33 D. Steffens, M. Lersch, A. Rosa, C. Scher, T. Crestani, M. G. Morais, J. a. V. Costa and P. Pranke, *J. Biomed. Nanotechnol.*, 2013, **9**, 710–718.

34 T. Elakkiya, G. Malarvizhi, S. Rajiv and T. S. Natarajan, *Polym. Int.*, 2014, **63**, 100–105.

35 C. Kriegel, K. M. Kit, D. J. McClements and J. Weiss, *J. Appl. Polym. Sci.*, 2010, **118**, 2859–2868.

36 S. Alborzi, L.-T. Lim and Y. Kakuda, *LWT - Food Sci. Technol.*, 2014, **59**, 383–388.

37 S. Maretschek, A. Greiner and T. Kissel, *J. Control. Release*, 2008, **127**, 180–187.

38 K. Stephansen, I. S. Chronakis and F. Jessen, *Colloids Surfaces B Biointerfaces*, 2014, **122**, 158–165.

39 A. Domínguez, A. Fernández, N. González, E. Iglesias and L. Montenegro, *J. Chem. Educ.*, 1997, **74**, 1227–1231.

40 M. Gandhi, R. Srikar, a. L. Yarin, C. M. Megaridis and R. a. Gemeinhart, *Mol. Pharm.*, 2009, **6**, 641–647.

41 I. C. Liao, S. Y. Chew and K. W. Leong, *Nanomedicine (Lond).*, 2006, **1**, 465–471.

348 42 W. O. McClure and G. M. Edelman, *Biochemistry*, 1966, **5**, 1908–1919.

349 43 L. Stryer, *J. Mol. Biol.*, 1965, **13**, 482–495.

350